DE GRUYTER OPEN

DOI: 10.1515/eces-2016-0023 ECOL CHEM ENG S. 2016;23(2):333-346

Ewa ZALEWSKA[1]

HARMFULNESS OF *Septoria carvi* SYD. TOWARDS CARAWAY AND PREPARATIONS LIMITING OCCURRENCE OF FUNGUS

SZKODLIWOŚĆ *Septoria carvi* SYD. DLA KMINKU ZWYCZAJNEGO I PREPARATY OGRANICZAJĄCE WYSTĘPOWANIE GRZYBA

Abstract: *Septoria carvi* is an important pathogen of caraway. With the increasing occurrence of septoriosis of caraway during warm and humid growing seasons, the possibility of limiting the growth and development of the fungus was investigated. In vitro studies included 2 preparations of natural origin, *ie* Biosept Active, Beta-chikol and 12 fungicides from different chemical groups, as well as one isolate K 1806 *S. carvi*, obtained from caraway. Tests were performed by poisoning the culture media. The percentage of inhibition of the growth of four- and eight-day-old fungus colonies on the medium with preparations in comparison to the control colonies was a measure of the toxic activity of the preparations. The effectiveness of Beta-chikol in limiting *S. carvi* colony growth was significantly higher than the efficiency of Biosept Active. All tested fungicides limited the growth of the fungus colony and their inhibitory effect increased with the concentration of the active ingredient in the medium. The most effective preparation *in vitro* was Signum 33 WG, belonging to the strobilurin compounds. *In vivo* studies investigated the effect of Beta-chikol, Biosept Active and Dithane NeoTec 75 WG on the health of caraway plants. The occurrence of septoriosis was determined basing on the presence of disease symptoms and the mycological analysis of diseased plants. A significant reduction of septoriosis symptoms on plants and plant colonization by the fungus was caused by Biosept Active and Dithane NeoTec 75WG.

Keywords: caraway, septoriosis, preparations of natural origins, fungicides

Introduction

Numerous species of fungi *Septoria* genera are very dangerous pathogens of plants [1-3]. *Septoria carvi* causes septoriosis of caraway in the form of necrotic spots on all above-ground parts of plant [4]. The infected plants die and the schizocarps are the first source of infection for the next generations of plants [4]. The fungus occurs with varying intensity in different growing periods, which is related to the requirement of life.

The optimum temperature for mycelial growth of *S. carvi* ranges from 20 to 25°C and 25°C for sporulation. At the temperature of 30°C the growth is much slower, and at 0 and 5°C the growth of the mycelium is minimal. Therefore, increased occurrence of *S. carvi* on caraway is noted in vegetation periods of high temperature and high air humidity.

In the Lublin region, such conditions are usually at the beginning or in the first half of June, which is conducive to the epidemic occurrence of septoriosis of caraway at the

[1] Department of Phytopathology and Mycology, University of Life Sciences in Lublin, ul. S. Leszczyńskiego 7, 20-069 Lublin, Poland, phone +48 81 524 81 42, fax +48 81 524 81 42, email: ewa.zalewska@up.lublin.pl

beginning of flowering [4]. In view of an increased economic importance of septoriosis of caraway in European countries [4-6], it is important to know the factors limiting the growth and development of the pathogen.

The aim of the present study was to investigate the effect of preparations of natural origin and fungicides on *S. carvi* in *in vitro* and *in vivo* conditions.

Material and methods

Effect of preparations *in vitro*

The study was conducted in 2010-2012. Two preparations of natural origin, *ie* Biosept Active and Beta-chikol and 12 fungicides of different chemical groups were included in the study. The list of the tested preparations according to the recommendations of Plant Protection for the years 2008/2009 and 2010/2011 is shown in Table 1. The isolate of *S. carvi* K 1806 (Fig. 1) obtained from caraway plants with symptoms of septoriosis (Figs. 2 and 3) and originated from our own professional collection was selected for study [4].

Table 1

Characteristic of examined preparations

No	Preparations	Name of active ingredient and its content in the preparations [%] or [g/dm^3]	Producer
1	Biosept Active	33% extract from grapefruit	Cintamani
2	Beta-chikol	20 g/dm^3 chitosan	Gumitex Poli-Farm
3	Amistar 250 SC	250 g/dm^3 - azoxystrobin	Syngenta LTD
4	Bravo 500 SC	500 g/dm^3 - chlorothalonil	Syngenta LTD
5	Captan 50 WP	50% - captan	ARYSTA LS
6	Curzate M 72,5 WP	4.5% - cymoksanil; 68% - mancozeb	DU PONT
7	Curzate Cu 49,5 WP	4.5% - cymoksanil; 45% - copper oxichloride	AZOT
8	Dithane NeoTec 75 WG	75% mancozeb	Dow AgroSciences
9	Domark 100 EC	100 g/dm^3 tetraconazole	ISAGRO
10	Gwarant 500 SC	500 g/dm^3 chlorothalonil	ARYSTA
11	Sadoplon 75 WP	75% thiuram	AZOT
12	Signum 33 WG	26.7% boksalide (anilide) 6.7% pyraclostrobin (strobilurine)	BASF
13	Topsin M 500 SC	500 g/dm^3 tiophanate methyl	NIPPON SODA
14	Zato 50 WG	50% trifloxystrobin	BAYER AG

This isolate was tested previously in terms of the requirements of life and pathogenicity. The tests were performed in two series, using the method of poisoning the culture medium and inoculating the fungal inoculum on them [7]. The effect of Biosept Active on *S. carvi* was tested at the concentrations 0.05, 0.1, 0.2 and 0.3, while Beta-chikol at 0.01, 0.025, 0.05 and 0.1%. The above mentioned concentrations of each of preparation are tested in accordance with earlier studies carried by Machowicz-Stefaniak and Zalewska [7] in relation to other pathogenic fungi. This mean indicates the species of fungi which growth and sporulation may be limited by Biosept Active and Beta-chikol. The fungicides were tested at the concentrations 1, 10, and 100 g·cm^{-3} a.i. with the aim of determining an approximate ED_{50} dose and including them within one of four groups of fungicidal activity [7]. The experiments *in vitro* were established and conducted as described by Machowicz-Stefaniak and Zalewska [7].

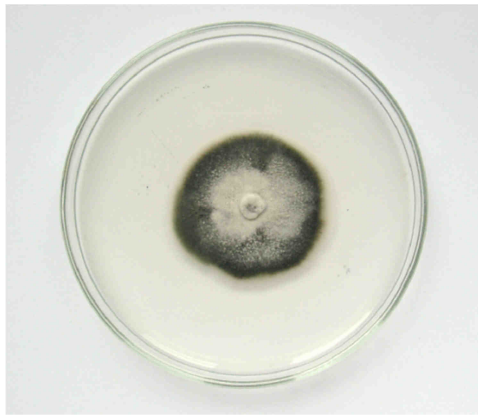

Fig. 1. 14-day-old colony of *S. carvi* isolate K 1806 on the PDA medium (Photo E. Zalewska)

Fig. 2. Septoriosis of caraway plants (Photo E. Zalewska)

Fig. 3. Spots on the leaf of caraway caused by *Septoria carvi* (Photo E. Zalewska)

The effect of preparations was estimated and measured as a percentage of inhibition of 4- and 8-day-old colonies of *S. carvi* growing on the medium with preparations in relations to the control colonies [7 according to quoted literature]. The microscopic observations of morphological structures of the fungus were conducted after 4 and 8 days of culture on the medium with an addition of the preparations. In the case of no growth of *S. carvi* on the medium with an addition of the preparations the type of the toxic activity on the fungus was determined [7].

Effect of preparations *in vivo*

The efficiency of preparations Biosept Active at the concentration 0.1%, Beta-chikol at 2%, *ie* and Dithane NeoTec 75 WG 0.3% at the concentrations recommended by producer, was tested. The control combinations consisted of plants not treated with any preparations, but only running water was used.

The studies were conducted on the experimental field in Motycz near Lublin, where caraway has been cultivated since 2001 year and septoriosis has been observed more often. 16 rows of 4 m each were marked out on the field and they were sown with caraway schizocarps, Konczewicki variety. One hundred plants grew in each row. Each of the preparations was tested on 400 plants grown in four randomly selected rows (4x100), which were treated as four replicates. The distance between rows was 45 cm.

The experiment was established in the second half of April 2011 and continued until harvest, *ie* until mid-July 2012. Schizocarps of caraway before sowing were soaked for 20 minutes in each of the three preparations tested at the concentrations mentioned above. Schizocarps treated only with clean water constituted the control. In the first year of study the observations of plants healthiness were made on plots at the phase of 6-week-old seedlings, which was at the end of June and then at full growing season, *ie* at the end of August. In the first year of cultivation, no signs of septoriosis were observed but in mid-September a preventive spray was applied, then in the second year of cultivation the protection was continued, spraying the plants since May 2, 2012.

Subsequent spraying was performed every 10-12 days with the last spraying on 21 June 2012. In 2012 six sprays with each preparation used in the full program were performed. Each time 2.5 dm^3 of each of the tested preparations was used for the spraying. In the control combination running water was used. Procedures were performed using a hand sprayer of KWAZAR company. After winter, *ie* on 24 April and before each treatment, the plants` health was determined.

The efficiency of preparations was estimated basing on the occurrence of septoriosis symptoms and the mycological analysis of plants. Septoriosis was evaluated on the basis of a 6-degree scale: 0^o - lack of septoriosis, 1^o - infection from 1 to 5% of leaf surface, 2^o - infection from 6 to 25% of leaf surface, 3^o - infection from 26 to 50% of leaf surface, 4^o - infection from 51 to 75% of leaf surface and finally 5^o - 76% to 100% surface of leaves showed septoriosis. After that, the infection index according to Patkowska [8] was estimated. At the time of flowering and at the beginning of schizocarps ripening and before harvest the mycological analysis of the above-grounds parts of caraway (leaves, stems, umbels and schizocarps) was carried out to determine their colonization by *S. carvi* [4]. The results were related to weather conditions on the basis of meteorological data for the Lublin area, obtained from the meteorological station in Radawiec using the data published on-line [http://www.weatheronline.pl] (Table 2).

Table 2

Comparison of average value of monthly temperature of air and rainfalls with average many years in vegetation periods in 2011-2012

Month	Means of the years 1963-1992		Difference of mean air temperature in comparison with means of the years		Percentage of the average annual rainfalls	
	air temperature [ºC]	rainfalls [mm]	2011	2012	2011	2012
January	–3.8	26.2	–2.6	1.7	120.99	164.88
February	–2.9	27.3	–1.8*	–4.65*	95.24	70.33
March	1.1	27.1	1.7	3.5	28.71	94.83
April	7.4	40.4	3.7	1.95	85.40	77.23
May	13.3	54.4	0.1	0.75	100.73	59.93
June	16.5	68.9	1.6	0.8	114.95	60.38
July	18.0	78.3	0.55	3.0	218.01	53.38
August	17.1	73.7	1.25	1.65	42.47	60.11
September	12.9	47.3	1.95	2.2	11.20	81.18
October	7.8	41.0	0.25	0.15	66.34	209.2
November	2.5	40.9	–0.2*	2.8	2.44	55.26
December	–1.4	33.8	2.9	–2.8*	112.78	87.57

*temperature lower than mean of the years

Results

Effect of preparations *in vitro*

Studies on the possibility of limiting the growth of *S. carvi* showed that the percentage of inhibition of the growth of 4-day-old colonies of the fungus was significantly higher on the medium with an addition of Biosept Active than in control, independently of the concentration of the preparation (Table 3). The percentage of growth inhibition of the fungus colony by Biosept Active at the concentration of 0.1, 0.2 and 0.3% did not differ significantly, but this percentage was significantly higher than at the concentration of 0.05% (Table 3). Biosept Active, independently of the concentration, inhibited the growth of 8-day-old colonies of *S. carvi* more strongly, than 4-day-old ones (Table 3). The most effective in reducing the growth of 8-day-old colonies of *S. carvi* was Biosept Active at the concentrations of 0.3 and 0.2%, and the least effective at the concentration of 0.05% (Table 3).

Table 3

Impact of the preparations of natural origin on the growth of *S. carvi* colony

Concentration [%] / Preparations	0.05	0.1	0.2	0.3	Control	LSD
	Percent of inhibition of the colony growth					
Biosept Active effect after 4 days	41.07 b	50.0 a	50.0 a	50.0 a	0.0 c	3.4909
effect after 8 days	45.54 c	57.59 b	59.82 ab	63.40 a	0.0 d	4.2423
Beta-chikol	0.01	0.025	0.05	0.1	Control	LSD
effect after 4 days	37.75 b	71.43 a	75.0 a	78.57 a	0.0 c	11.912
effect after 8 days	58.93 b	83.03 a	81.25 a	89.29 a	0.0 c	9.5517

Values marked with the same letter do not differ significantly, $p \leq 0.05$

The result of Beta-chikol effect on the growth of 4-day-old as well as 8-day-old colonies of *S. carvi*, independently of the concentration of the preparations, was significantly lower than in control. The percentage of growth inhibition of 4- and 8-day-old colonies at the concentration of 0.025%, 0.05% and 0.1% did not differ significantly but it was significantly higher than at the concentration of 0.01% (Table 3).

The conducted observations showed that in contrast to the control colony, the colonies of *S. carvi* growing in the presence of Biosept Active and Beta-chikol produced poor and compact aerial mycelium with numerous dark-brown and fat hyphae. On the course of hyphae numerous chlamydospores were observed.

Table 4

Effect of fungicides tested on growth inhibition of 4-days-old colonies of *S. carvi*

Fungicides *	**Percent of inhibition in relation to a.i. concentration [g·cm^{-3}]**		
	1	**10**	**100**
I			
Signum 33 WG	100 i	100 i	100 i
Zato 500 WG	56.36 g	61.81 g	56.36 g
Domark 100 EC WP	54.54 fg	78.18 h	100 i
Curzate M 72,5 WP	52.72 efg	100 i	100 i
II			
Sadoplon 75 WP	34.54 cd	100 i	100 i
Dithane NeoTec 75 WG	43.63 def	61.81 g	100 i
Amistar 250 SC	43.63 def	56.36 g	61.81 g
Topsin M 500 SC	3.64 b	56.36 g	100 i
Bravo 500 SC	41.81 de	52.73 efg	56.36 g
Gwarant 500 SC	38.18 d	50.91 efg	54.54 fg
III			
Captan 50 WP	–16.36 a	–14.54 a	76.36 h
IV			
Curzate Cu 49,5 WP	0.0 b	–1.81 b	25.45 c
Control	0.0 b	0.0 b	0.0 b
LSD	6.213		

Values marked with the same letter do not differ significantly; *fungicides compared according to fungicidal activity group

As the result of studies the tested fungicides were included within four groups of fungicidal activity towards *S. carvi* (Tables 4 and 5). Significantly the highest percentage of limiting the growth of 4-day-old colonies, independently of the concentration was caused only by the preparation Signum 33WG. However, Curzate M 72.5 WP inhibited the growth of colony in 100% at the concentration of 10 and 100 g·cm^{-3}, and Domark 100 EC at the concentration of 100 g·cm^{-3}. Therefore, the above mentioned preparations and Zato 500 WG were classified within Group I of toxic activity, *ie* within the substances of very strong fungicidal activity towards *S. carvi* (Table 4). Within Group II of toxic activity, *ie* within the substances of strong fungicidal activity, 6 preparations were included (Table 4). Among those preparations 100% inhibition of fungal colony growth was caused by Sadoplon 75WP at the concentration 10 and 100 g·cm^{-3}, Dithane NeoTec 75 WG and Topsin 500 S.C. at 100 g·cm^{-3} (Table 4). Within Group III of toxic activity, *ie* the substances of moderate fungicidal activity towards *S. carvi*, Captan 50 WP was included, which at the concentration of 1 and 10 g·cm^{-3} stimulated the growth of the pathogen. Only Curzate Cu 49.5 WP, which at the concentration of 10 g·cm^{-3} stimulated the growth of the

fungus colony, was included within the fourth group of fungicidal activity, *ie* within the substances of poor fungicidal activity (Table 4).

Table 5

Effect of fungicides tested on growth inhibition of 8-days-old colonies of *S. carvi*

Fungicides*	**Percent of inhibition in relation to a.i. concentration [g·cm^{-3}]**		
	1	**10**	**100**
I			
Signum 33 WG	79.41 lm	88.24 mn	100 n
Zato 500 WG	56.86 ijk	58.82 ijk	55.88 hij
Domark 100 EC	55.88 hij	80.39 lm	88.23 mn
Dithane NeoTec 75WG	54.9 ghij	63.72 ijk	100 n
Amistar 250 SC	52.94 ghi	53.92 ghij	60.78 ijk
II			
Sadoplon 75 WP	25.49 cd	88.23 mn	100 n
Curzate M 72,5 WP	44.12 fgh	80.39 lm	100 n
Topsin M 500 SC	0.49 b	68.63 kl	100 n
III			
Captan 50 WP	–32.25 a	–31.37 a	65.69 jk
IV			
Bravo 500 SC	19.61 c	21.57 c	43.14 fg
Gwarant 500 SC	18.63 c	34.31 def	38.24 fg
Curzate Cu 49,5 WP	0.0 b	–0.98 b	27.25 cde
Control	0.0 b	0.0 b	0.0 b
LSD	6.3833		

Explanations like in Table 3

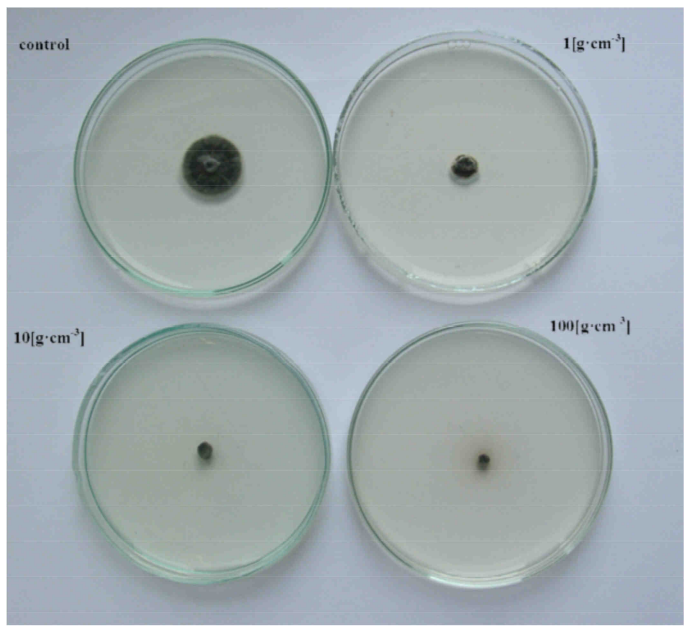

Fig. 4. 8-day-old colonies of *S. carvi* K 1806 on the PDA medium with Signum 33 WG (Photo E. Zalewska)

After 8 days of the effect, the toxic activity of fungicides towards *S. carvi* decreased. Five fungicides belonged to the first group of toxic activity, including Signum 33 WG, which inhibited the fungus growth the most strongly, independently of the concentration (Table 5, Fig. 4). In II group of toxic activity there were three preparations, which at the concentration of 100 g·cm^{-3} c.a. completely inhibited the growth of the fungus colony. Captan 50 WP, like after 4 day of activity, was in III group of toxic activity and also stimulated the growth of *S. carvi* colony at the concentration 1 and 10 g·cm^{-3}. Bravo 500 S.C., Gwarant 500 S.C. and Curzate Cu 49,5 WP were included within IV group of toxic activity (Table 5). Independently of the concentration of c.a., significantly the highest percentage of inhibition of 4 and 8-day-old colonies of *S. carvi* was caused by Signum 33WG, Curzate M 72,5 WP, Sadoplon 75WP, Domark 100EC and Dithane NeoTec 75WG (Table 6, Fig. 4).

Table 6

Effect of fungicides on the growth of *S. carvi* colony independently of active ingredients concentration (mean of all studied concentrations)

Fungicides	**Percent of inhibition**	
	after 4 days	**after 8 days**
Signum 33 WG	100 a	82.2 a
Curzate M 72,5 WP	84.2 b	74.8 b
Sadoplon 75 WP	78.2 bc	71.2 b
Domark 100 EC	77.6 bc	74.8 b
Dithane NeoTec 75 WG	68.5 d	72.9 b
Zato 50 WG	58.2 e	57.2 c
Amistar 250 SC	53.9 ef	55.9 c
Topsin M 500 SC	53.3 ef	56.5 c
Bravo 500 SC	50.3 f	28.1 d
Gwarant 500 SC	47.9 f	30.4 d
Captan 50 WP	15.2 g	0.7 f
Curzate Cu 49,5 WP	7.9 h	8.8 e
Control	0.0 i	0.0 f
LSD NIR	6.213	4.3139

Values marked with the same letters do not differ significantly

Table 7

Toxic activity of fungicides on *S. carvi*

Fungicides	**Toxic activity**		
	1 g·cm^{-3}	**10 g·cm^{-3}**	**100 g·cm^{-3}**
Amistar 250 SC	+	+	+
Bravo 500 SC	+	+	+
Captan 50 WP	++	++	+
Curzate M 72,5 WP	+	+	_
Curzate Cu 49,5 WP	0	++	+
Dithane NeoTec 75 WG	+	+	_
Domark 100 EC	+	+	+
Gwarant 500 SC	+	+	+
Sadoplon 75 WP	+	+	_
Signum 33 WG	+	+	_
Topsin M 500 SC	+	+	_
Zato 50 WG	+	+	+

- fungicidal activity; + fungistatic activity; ++ stimulating activity; 0 the lack of activity

Among the tested fungicides, Curzate M 72,5WP, Dithane NeoTec 75WG, Sadoplon 75WP, Signum 33WG and Topsin M 500SC showed fungicidal activity towards *S. carvi* (Table 7).

Basing on macroscopic and microscopic studies it was shown that a lot of fungicides tested limited sporulation of the pathogen. Pycnidia and conidia were observed sporadically at the lowest studied concentration of c.a. at 1 $g \cdot cm^{-3}$ of preparations: Amistar 250 SC, Domark 100 EC, Curzate M 72,5 WP and Captan 50 WP. Moreover, hyphae of *S. carvi* at the presence of the other fungicides were deformed and produced chlamydospores but sclerotia and pycnidia were formed very rarely (Fig. 5). However, after dissection of the pycnidia no conidia were observed.

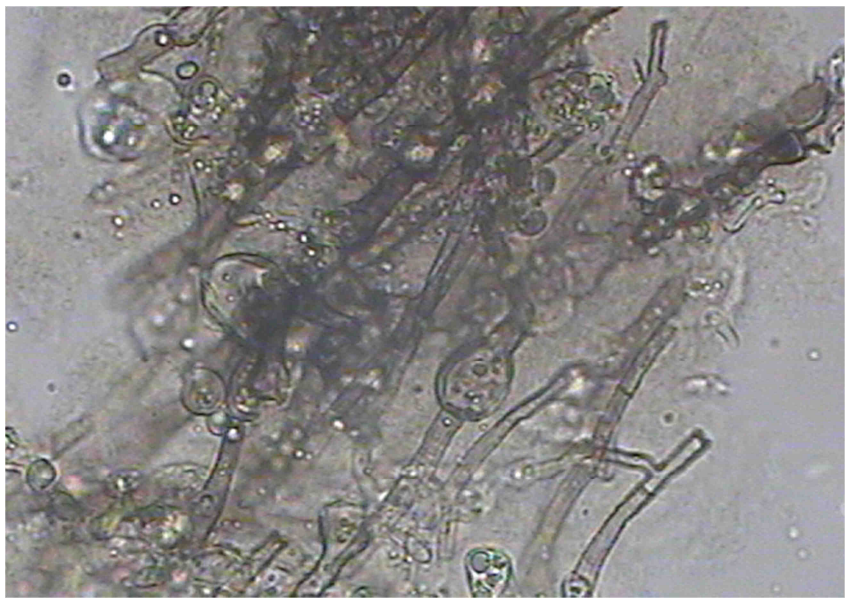

Fig. 5. Degeneration of *S. carvi* hyphae and chlamydospores on the PDA medium with Signum 33 WG (magnification 500x) (Photo E. Zalewska)

Effect of preparations *in vivo*

Observations of plants' healthiness after sowing the schizocarps treated with the studied preparations of natural origin, preparation Dithane NeoTec 75 WG and plants from the control combination, showed the lack of diseases symptoms on plants in the first year of cultivation. After preventive spraying of plants with each of the studied preparations in the middle of September, the plants characterized by good growth had a big rosette of leaves and their height was up to 15-20 cm. The next observation made after overwintering of plants, *ie* on 24 April, 2012 indicated the presence of individual symptoms of septoriosis on caraway leaves. The lowest infection index of plants was found in the combination with fungicide Dithane NeoTec 75WG, while the biggest infection index was found in the control combination but those values did not differ significantly (Table 8).

The infection index of plants with symptoms of septoriosis in the combination with Biosept Active and Beta-chikol was, respectively, 1.05 and 1.49 and it was significantly

lower than in the control combination but it was bigger than in the combination with Dithane NeoTec 75WG (Table 8). After the following three sprayings the infection index of leaves caused by *S. carvi* increased gradually. However, in the combination with Biosept Active and Dithane NeoTec 75 WG those indexes did not differ significantly and they were significantly lower than in the control combination (Table 8). However, in the combination with Beta-chikol they were significantly higher than in the combination with Biosept Active and Dithane NeoTec 75 WG and they did not differ significantly from the index in the control combination (Table 8). In late June the infection index in the control combination rose to 30.45 and it was significantly higher than in the other combinations of the experiment (Table 8). Among the three preparations tested the most effective was Dithane NeoTec 75WG - infection index 10.15 and this value was significantly lower than the infection index in the combination with Biosept Active - 18.08 and Beta-chikol - 20.33 (Table 8).

Table 8

The indicators of infection of caraway leaves by *Septoria carvi* during the growing season - spring/summer 2012

Date of observation and spraying / **Combination**	**24.04. 2012**	**02.05. 2012**	**13.05. 2012**	**24.05. 2012**	**02.06. 2012**	**11.06. 2012**	**21.06. 2012**
Biosept Active	1.05 a	1.21 a	1.63 a	3.89 b	8.96 b	8.85 b	18.08 b
Beta-chikol	1.49 a	1.25 a	1.55 a	9.99 a	14.24 a	18.29 a	20.33 b
Dithane NeoTec 75WG	0.71 a	0.88 a	1.84 a	5.46 b	9.44 b	9.79 b	10.15 c
Control	2.14 a	0.95 a	1.96 a	9.81 a	16.58 a	22.03 a	30.45 a
LSD	2.756	2.3982	2.555	3.6138	4.0217	4.9385	4.9603

Values marked with the same letter do not differ significantly, $p \leq 0.05$

During the observation of the healthiness of caraway plants in the first year of cultivation, the high percentage of rainfall norm in summer months, *ie* from May to August, was found. Moreover, the air temperature was higher than the long-term average air temperature. In the second year of cultivation, during the growth, flowering and ripening of schizocarps, the percentage of the norm of rainfall was low and the air temperature was higher than the long-term average (Table 2).

The mycological analysis of above-ground parts of caraway plants showed their colonization by various species of fungi (Table 9). *Septoria carvi* was isolated from all parts of plants in all combinations of the experiment. However, this fungus was obtained more often from the leaves and leaf petioles of plants treated with Biosept Active and Beta-chikol.

The proportion of isolates of this species among the fungi obtained from these parts of plants was 62.93 and 83.87% respectively in combination with Biosept Active but 72.73 and 41.67% in the combination with Beta-chikol (Fig. 6). Moreover, numerous isolates of this fungus were obtained from the leaves and petioles of plants in the control combination. In the combination with Dithane NeoTec 75 WG only single isolates of *S. carvi* were obtained from petioles while from the leaves they accounted for approximately 30% (Table 9, Fig. 6).

Table 9

Fungi isolated from various organs of caraway *Carum carvi* L. plants treated with Beta-chikol, Biosept Active, Dithane NeoTec 75 WG and from control combination in 2012

Fungi	Number of isolates								Total	
	Preparations (term of isolation)									
	Bio-sept Active I	Bio-sept Active II	Beta-chikol I	Beta-chikol II	Dithane NeoTec 75 WG I	Dithane NeoTec 75 WG II	Control I	Control II	I	II
***Septoria carvi* Syd.**	**53**	**4**	**39**		**6**	**10**	**47**	**23**	**145**	**37**
Other fungi										
Acremonium strictum W. Gams	2				2		8		12	
Alternaria alternata (Fr.) Keissl.	7	127	8	108	19	117	55	90	89	442
Alternaria radicina Meier Drechsler et E.D. Eddy	37		32		8		6		83	
Bipolaris spp.		2								2
Botrytis cinerea Pers.			2				2		4	
Chaetomium globosum Kunze					2				2	
Cladosporium cladosporioides (Fresen.) G.A. de Vries	4		2		10		4		20	
Colletotrichum dematium (Pers.) Grove	2		2				2		6	
Colletotrichum gloeosporioides (Penz.) Penz. et Sacc.						11				11
Epicoccum purpurascens Ehrenberg	11		8	2			5		24	2
Fusarium oxysporum Schltdl.		10		1						11
Fusarium sporotrichioides Sherb.		7	4	1		2		25	4	35
Penicillium cyclopium Westling	2		3						5	
Phoma exigua var *exigua* Desm.					2				2	
Sclerotinia sclerotiorum (Lib.) de Bary				9			2		2	9
Total other fungi	**65**	**146**	**61**	**121**	**43**	**130**	**84**	**115**	**253**	**512**
Total	118	150	100	121	49	140	131	138	398	549

I - first term of isolation during flowering and ripening of schizocarps; II - second term of isolation during harvest time

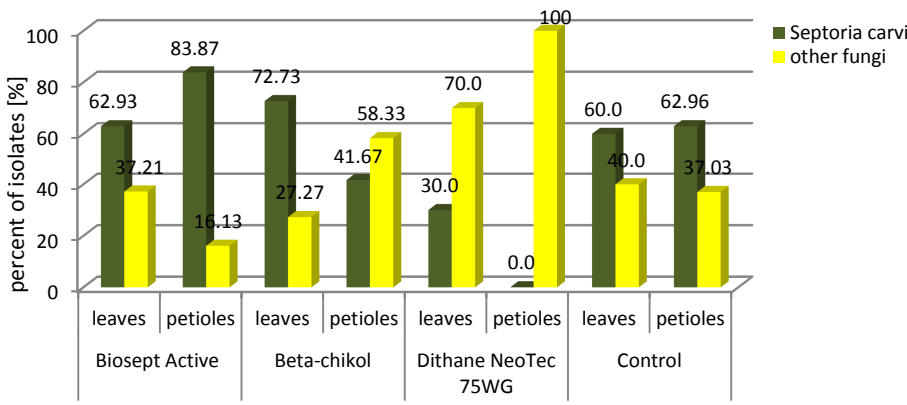

Fig. 6. Participation of *S. carvi* isolates among the fungi obtained from leaves and petioles of caraway during the time of flowering and ripening of schizocarps

In the second term of isolation, *S. carvi* was obtained with less intensity than during the first one. However, numerous isolates of other fast-growing and dark-sporulating species of fungi were obtained (Table 9).

Discussions

Research on the disease of caraway, conducted since 2001, indicate a high threat of *S. carvi* for plant. This is confirmed by isolating the fungus from the above-ground parts of plants in final years of vegetation [4].

In connection with periodically increased occurrence of septoriosis of caraway on plants in the second year of cultivation and on plants in the first year of growth, all information about the possibility of limiting the pathogen is very valuable. The study *in vitro* on the possibility of limiting the growth and development of *S. carvi* colony using Biosept Active showed a significant inhibitory effect of this preparation. Moreover, it was shown that the efficiency of Biosept Active increased slightly with an increased content of active ingredient in the medium. However, this effect was insufficient, since although biologically active substances are present in the preparation, including endogenous flavonoids inhibiting spore germination and growth of vegetative hyphae and germination of spores [7], the growth of fungus was observed. A similar effect of Biosept Active was observed towards *P. diachenii*, which limited the growth of the fungus colony only in the first days of the experiment. However, with respect to other pathogenic species such as *Colletotrichum gloeosporioides* and *C. dematium* a strong reduction of colony growth on PDA medium containing Biosept Active was observed [7, 9]. The study showed a high inhibitory effect of Beta-chikol *in vitro*, far exceeding the effect of Biosept Active towards *S. carvi*. Similar effects were also observed in the previous studies on *P. diachenii*. However, the studies carried by Machowicz-Stefaniak and Zalewska [7] and Pieta et al [9] showed a much weaker effect of Beta-chikol towards *C. dematium* and *C. gloeosporioides* than Biosept Active. The results of the present studies *in vitro* and previous studies with respect to other pathogenic fungi indicate that the effect of natural preparations on phytopathogenic fungi varied and they should be examined individually for each species [7, 9].

The studies *in vitro* indicate differences in the size and development of *S. carvi* colonies on media containing various concentrations of the active substance of fungicides tested. Furthermore, it was observed that the percentage of growth inhibition of the pathogen colonies increased with an increased content of active fungicides tested. Several fungicides tested even showed fungicidal properties reflected not only by the loss of vigor of spores but the mycelium of *S. carvi* too. Fungicides with such properties are highly desirable in the context of controlling the pathogenic fungi [7, 10].

The above effect, like towards *P. diachenii*, depended on the concentration of active substances in the medium, but not on the time of effect on the fungus colony. This relation was indicated earlier towards *Colletotrichum dematium* and *Phomopsis viticola* [7, 10].

It was found that compounds which determine the lethal effect on the vitality of *S. carvi* are boksalide and pyraclostrobin, mancozeb, thiuram and methl-thiophanate found, respectively, in preparations Signum 33 WG, Dithane NeoTec 75WG, Curzate M 72,5 WP, Sadoplon 75 WP and Topsin M 500 SC.

The present studies indicate that the growth and development of *S. carvi* is limited by a wide range of chemical compounds, which are recommended for the control of

septoriosis of many vegetable species. The efficacy of these compounds was previously shown for other fungi pathogenic towards caraway. It was observed that the preparation which most limit the growth and development of *S. carvi* was Signum 33 WG. A similarly, high effect of this preparation was shown in the studies Mačkinaitė [6] with respect to various fungi isolated from caraway schizocarps. However, in the studies carried by Machowicz-Stefaniak and Zalewska [7], mancozeb was a highly effective compound in limiting the growth and development of *Colletotrichum dematium* and *Phomopsis diachenii.* Moreover, mancozeb showed a destructive effect on the morphological structures of these pathogens. A high fungicidal activity of macozeb was shown for fungi infecting orchard plants *ie* for *Phomopsis viticola* [10] and towards various species of fungi infecting herbs, *ie Phoma anethi, Alternaria alternata* and *Fusarium* spp. [6].

Changes in appearance of the colony and hyphae of fungus were observed on the medium containing active ingredients of the preparations limiting the size of *S. carvi* colony. The production of sclerotia, aggregates of pycnidia without spores, chlamydospores and barren stands of fungal hyphae indicate an adverse effect of preparations towards *S. carvi.* The formation of overwintering forms, *ie* sclerotia and chlamydospores, rarely occurring in *Septoria* spp., demonstrates a negative effect of the preparations on fungi [7].

Despite a significant reduction of *S. carvi* growth *in vitro* by preparations of natural origin, their effect on limiting the occurrence of caraway septoriosis *in vivo* was unsatisfactory. Although the values of infection index of plants were small, and the plants grew and stained well, they were inhabited by *S. carvi*, which was shown by the mycological analysis in the second year of cultivation. It seems that the appearance of the first symptoms of septoriosis at the beginning of the growing periods on biennials plants in April 2012 was strongly affected by weather conditions occurring in the previous vegetation period. Despite the lack of disease symptoms on plants in the first year of vegetation, rainfall occurring in the summer months and high air temperature exceeding the average long-term values probably caused the occurrence of latent infection of plants and overwintering of the pathogen in plants tissues. This is possible because the hyphae of the fungus can grow at the temperature of 0ºC.

A much smaller number of *S. carvi* isolates obtained from caraway plants during the harvest time as compared to the time of flowering results from the weak competitive properties of *S. carvi* towards other species of fast-growing fungi which occur commonly on plants during the end of their vegetation period.

It was indicated that in natural conditions the effectiveness of Biosept Active during the intensification of septoriosis on plants exceeded, otherwise than in laboratory conditions, the effectiveness of Beta-chikol. In addition, the protective effect of Biosept Active in the conditions of the present studies was not worse than the protective influence of Dithane NeoTec 75 WG. Therefore, the research on the practical application of preparations of natural origin will be continued.

Conclusions

1. *Septoria carvi* is a very frequently occurring species pathogenic towards caraway and it should be controlled using control spraying.
2. *In vitro* growth and development of *S. carvi* are limited by preparations of natural origin and fungicides.

3. Among the preparations of natural origin the inhibiting effect of Beta-chikol *in vitro* towards *S. carvi* was significantly higher than the efficiency of Biosept Active. However, in natural conditions the efficiency of Biosept Active in the period when septoriosis of caraway increased, exceeded the efficiency of Beta-chikol.
4. Most of the tested fungicides limited the growth and development of *S. carvi in vitro.* The effect of Signum 33 WG, Dithane NeoTec 75 WG, Sadoplon 75 WP, Curzate M 72,5 WP and Topsin M 500 SC was fungicidal but the most effective was Signum 33 WG.
5. Despite the limited occurrence of septoriosis on plants caused by preparations tested *in vivo*, plants were inhabited by *S. carvi*, which indicate difficulties in eliminating the major pathogen of plants.
6. Due to the varied effect of preparations on plants` pathogens, they should be tested individually for each species of fungi.

Acknowledgements

The studies were supported by Ministry of Science and Higher Education, grant No NN310449938.

References

[1] Sutton BC. The Coelomycetes. Fungi Imperfecti with Pycnidia, Acrevuli and Stromata. Comm Mycol Inst. Kew Surrey. England; 1980.
[2] Quaedvlieg W, Verkley GJM, Shin H-D, Barreto RW, Algenas AC, Swart WJ, et al. Sizing up Septoria. Stud Mycology. 2013;75(1):307-390. DOI: 10.3114/sim0017.
[3] Verkley GJM, Quaedvlieg W, Shin H-D, Crous PW. A new approach to species delimination in Septoria. Stud Mycology. 2013;75(1):213-305. DOI: 10.3114/sim0018.
[4] Machowicz-Stefaniak Z, Zalewska E. Biodiversity of fungi inhabiting various parts of carway (Carum carvi L.). EJPAU. Horticulturae. 2008;11(1)#21. http://www.ejpau.media.pl/volume11/issue1/art.-21.html.
[5] Bedlan G. Septoria carvi an Kümmel. Gemüse. 2005;11:25. http://www.gemuese-online.de/Archiv/PDF-Archiv/Septoria-carvi-an-Kuemmel,QUlEPTQ3MTQxMiZNSUQ9MTA5Njk1.html.
[6] Mačkinaitė R. Potential pathogens of common caraway (Carum carvi L.) seeds and search for measures suppressing their spread. Žemdirbystė=Agriculture. 2012;99(2):179-188. http://www.lzi.lt/tomai/99(2)tomas/99_2_tomas_str9.pdf.
[7] Machowicz-Stefaniak Z, Zalewska E. Occurrence of Colletotrichum dematium on selected herbs species and preparations inhibiting pathogen's growth and development in vitro. Ecol Chem Eng S. 2011;18(4):465-478. https://www.researchgate.net/publication/303920041.pdf.
[8] Patkowska E. Effect of Miedzian 50 WP and grapefruit extract on the healthiness and communities of soil microorganisms of pea (Pisum sativum L.). Acta Sci Pol Hortorum Cultus. 2014;13(3):23-33. http://www.hortorumcultus.actapol.net/volume13/issue3/13_3_23.pdf.
[9] Pięta D, Patkowska E, Pastucha A. Influence of biopreparations on growing and development of some pathogenic fungi. Acta Sci Pol Hortorum Cultus. 2004;3(2):171-177. http://www.hortorumcultus.actapol.net/pub/3_2_171.pdf.
[10] Król E. Influence of some chemicals on the variability of Phomopsis viticola Sacc. Spores. J Plant Prot Res. 2005;45(3):195-204. https://www.researchgate.net/publication/228480173.pdf.